Discerning the role of *bus-1* gene in susceptibility of *Caenorhabditis elegans* to pathogenic *Microbacterium nematophilum* via an inducible protein degradation protocol using Auxin.

By

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A thesis submitted in partial fulfillment of the requirements
for graduation with research distinction in the undergraduate
colleges of The Ohio State University.

The Ohio State University

Summer 2015

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Abstract

A host/pathogen interaction is dependent on many factors; this research focuses on susceptibility due to a host's genome. In *Caenorhabditis elegans*, a *Pax* gene encodes for a transcription factor (EGL-38) that regulates genes expressed in the hindgut of the nematode. This hindgut function is important for mediating the response to the bacterial pathogen *Microbacterium nematophilum*. When a *C. elegans* becomes infected by *M. nematophilum*, the worm experiences severe constipation, a deformed anal region (DAR), and stunted growth. *egl-38* mutants are resistant to *M. nematophilum*, whereas wild-type animals are sensitive. Gene expression microarray analysis of RNA from a wild type *C. elegans* verses *egl-38* mutants identified many genes that might be important for mediating the sensitivity. We tested these candidates using a combination of RNAi knock down, genetic *C. elegans* mutant experiments and gene expression studies. These experiments identified one EGL-38 regulated gene, *bus-1*, that is required for *M. nematophilum* sensitivity. We also confirmed that *bus-1* is specifically expressed in the hindgut. We have introduced BUS-1 protein (under control of a different promoter, *egl-5*) into an *egl-38* mutant *C. elegans* and found that it restores sensitivity to the mutant. These findings further support that the *bus-1* gene product is required for this host/pathogen interaction and that *bus-1* is a key EGL-38 target gene. However, what is not clear is whether *bus-1* plays a developmental role or a physiological role in mediating the host/pathogen response. In order to specifically target BUS-1, we are constructing an inducible protein degradation protocol using a plant hormone, Auxin. This system has been incorporated into yeast cultures and human cell lines and has the ability to induce the degradation of proteins tagged with a specific sequence within cells. We are generating plasmids to tag BUS-1 and observe the physiological response that occurs in the presence of *M. nematophilum* upon degradation of the *bus-1* gene product. With the generation of these plasmids, we will be able to further analyze the effect that *bus-1* has on this host/pathogen interaction.

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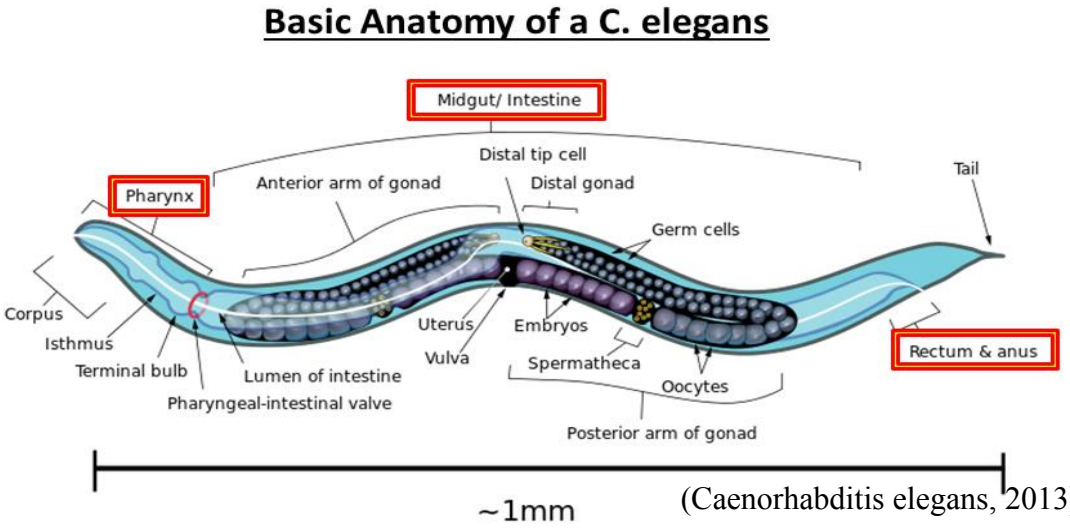
Introduction

This research aims to investigate the intricacies of a host and pathogen interaction in the model system of *Caenorhabditis elegans* and *Microbacterium nematophilum*. *C. elegans* are a species of nematode and an organism regularly used as a simple model system for biomedical research ("WormClassroom", 2013). *C. elegans* are easy to propagate, are able to self-fertilize in order to create genetic clones and grow from egg to adult within the span of three days. All of these characteristics along with the ability to generate hundreds of organisms in a short period of time, the low cost of maintaining the organisms and clear cuticle, which allows one to see the internal structures of the nematode, make *C. elegans* a popular model system. This project will use the vast knowledge already discovered about the *C. elegans* genome in order to discover the genomic players in this host/pathogen interaction.



(Caenorhabditis elegans, 2013)

M. nematophilum is a bacterial pathogen to *C. elegans* and some related nematodes. *M. nematophilum* is a gram negative bacterium which colonizes in the hindgut of the *C. elegans* and causes severe constipation, a distended anal region (DAR phenotype) and stunted growth. *M. nematophilum* was first discovered to have this effect in 1988 in Dr. Brenner's research laboratory when it contaminated plates of *C. elegans* (Darby, 2005). A host/pathogen interaction is dependent on many factors; this research focuses on susceptibility due to a host's genome. In *C. elegans*, a *Pax* gene encodes for a transcription factor (EGL-38) that regulates genes expressed in the hindgut. This hindgut function is important for mediating the response to the pathogen *M. nematophilum*.

egl-38 mutants are resistant to *M. nematophilum*, whereas wild-type animals are sensitive. It is important to note the chronological nature of this research, which has been distinguished as two separate aims. Aim one includes a functional genomic analysis of *C. elegans* in regards to susceptibility of *M. nematophilum,* including the support that one specific gene, *bus-1* and its protein product is a main player in the host/ pathogen interaction. Aim two focuses on creating and using an Auxin inducible protein degradation protocol to determine, physiologically, when *bus-1* is necessary to allow the *M. nematophilum* to colonize in the hindgut of the *C. elegans.* This research employs experiments to determine, specifically, which gene targets within the host's genome allow the pathogen to colonize in the hind gut of *C. elegans.* Data collected via reintroduction of *bus-1* protein product into a mutant *C. elegans* determined the gene *bus-1* and its protein product is sufficient to infect and induce a DAR phenotype in *C. elegans* in the presence of *M. nematophilum.*

RESEARCH AIM 1

This project will describe the steps taken in order to determine the components of the *C. elegans* genome that allow the *M. nematophilum* to colonize and infect the hindgut of the nematode. In order to determine the specific gene(s) and protein byproducts responsible for this interaction, we used gene expression microarray analysis, RNAi knock down and gene expression studies. The results of these experiments support *bus-1* as being a necessary factor in the host/pathogen interaction of *C. elegans* and *M. nematophilum.*

Aim 1 Methods

Gene expression microarray analysis

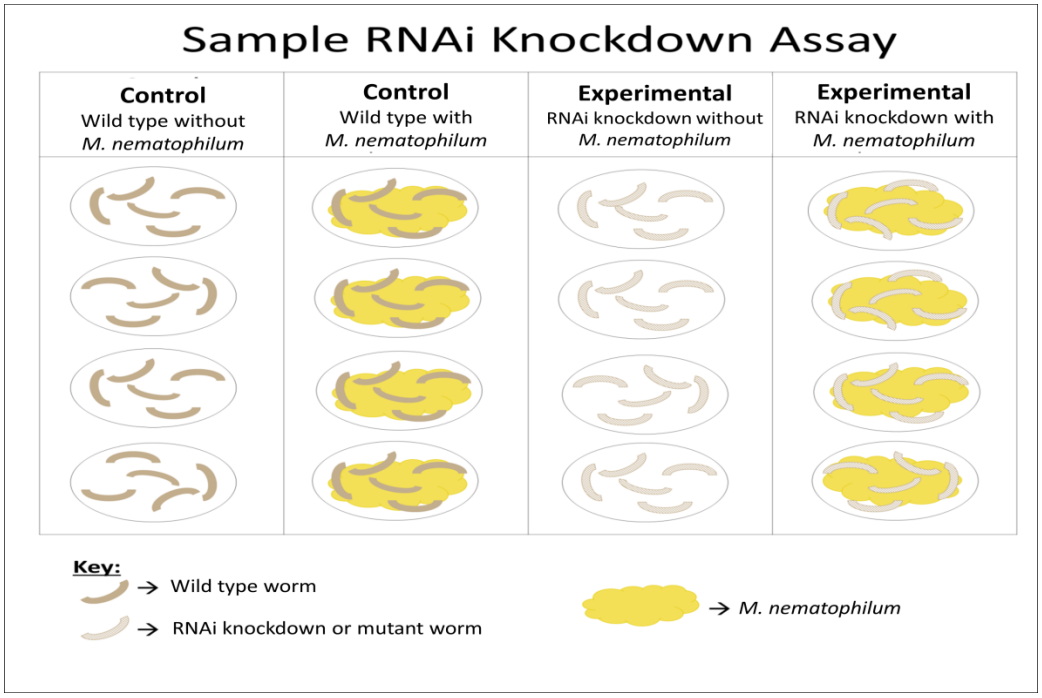
To establish, locate and silence the gene(s) responsible for the interaction of the *M. nematophilum* pathogen to the *C. elegans,* the Chamberlin lab carried out a gene expression microarray analysis (Jia, Johnson and Chamberlin, unpublished). RNA (in triplicate) was harvested from staged L4 worms using Trizol, and hybridized to affymetrix *C. elegans* GeneChip arrays. RNA was recovered from N2 (wild type), and two *egl-38* mutant strains, *egl-38(n578)* and *egl-38(sy294).* A cross reference of the three genotypes identified 33 genes with a significant ($p<0.01$, and two-fold change) decrease in abundance in *egl-38(n578)*, *egl-38(sy294)* or both compared to wild type. We hypothesized that one or more of these genes is/are responsible for mediating the host/pathogen interaction dependent on egl-38. From this list of genes, more experiments were employed in order to determine which specific genes are functionally important.

Mutant analysis and RNAi knockdown

The 33 genes identified in the microarray analysis were evaluated for existing mutant alleles available from the Caenorhabditis Genetics Center or other sources, and for clones targeting each gene available in the Ahringer *C. elegans* RNAi library. Mutant worms were evaluated for their sensitivity to *M. nematophilum* using a growth assay. The experiment compared each mutant to wild type worms on lawns of OP50 (*E. coli*) or *M. nematophilum* (grown in a 9:1 OP50:*M. nematophilum* lawn). The wild type worms on the OP50 lawns reach L4 or adult stage in three days, whereas the wild type worms on the *M. nematophilum* lawn exhibit slow growth and take longer than three days to reach adulthood, or arrest as larvae. L4 parents were plated onto each

plate on day 0. These parents were removed from the plate on day 1, and total number of eggs was counted. Two days later (day 3) the number of animals reaching L4 or older stage were counted. The proportion of worms that reached adulthood (L4 stage) were calculated by dividing the number of L4 adults (counted on day 3) by the number of eggs (counted on day 1). If the experimental populations on the *M. nematophilum* lawn grew statistically faster than the control on *M. nematophilum*, it could be inferred that the gene product knocked down is necessary for the host/pathogen interaction to occur. For the RNAi experiments, wild type animals were used in all assays and *E. coli* strains from the Ahringer RNAi library were used in place of OP50 for the knockdown condition. *M. nematophilum* bacteria exhibit resistance to Ampicillin (REF), and therefore can grow on RNAi feeding plates. Below is an example of a mutant or RNAi knockdown assay (Figure 1).

Figure 1.



Gene expression analysis

To characterize whether any of the EGL-38-dependent genes are expressed specifically in hindgut cells, we developed GFP reporter transgenes. This was done by designing primers which were used in polymerase chain reactions (PCR) to quickly and accurately replicate DNA. This DNA was then cloned into a plasmid next to the reporter gene GFP. Once this GFP clone was

created, it was injected into the nematodes to establish transgenic lines. These lines were evaluated using fluorescent microscopy to determine specifically where the gene is expressed in a wild type *C. elegans*. The reporter transgenes are transcribed within the nematodes and showed up as bright green under florescence. This allowed us to visually determine exactly where these genes are expressed; i.e. on the surface of the intestinal wall, in the intestinal wall, etc. Specifically, we were looking at the expression of these gene products within the hindgut of the *C. elegans.*

Aim 1 Results

The microarray analysis measured the transcription abundance of mutant alleles verses the transcription abundance of those same alleles within a wild type *C. elegans.* Both *egl-38(n578)* and *egl-38(sy294)* mutant *C. elegans* are resistant to *M. nematophilum*. An *egl-38(n578)* mutant has a very strong negative effect on EGL-38 function specifically in the egg-laying system and on *M. nematophilum* sensitivity, whereas an *egl-38(sy294)* mutant causes a more general negative effect of EGL-38 in all functions. Results of the microarray analysis can be seen below in Table 1 for the genes tested for M. nematophilum response.

Table 1.

Gene Name	Common Gene Name	Mutant Strain	egl-38(n578)[1]	egl-38(sy294)[1]
R03H4.6	bus-1	CB5674	0.296	0.272
ZK6.7	lipl-5	OK3581	0.48	0.427
K04A8.5	lipl-4	VC20551/g	0.466	0.385
R11G11.14	lipl-3	N/A	0.17	0.241

**Ratios present in table were calculated by dividing transcript abundance of each gene in worms bearing the mutant alleles by the transcript abundance of the same gene in a wild type worm.

Ratios calculated from the microarray analysis provided a list of genes that have decreased transcription in *M. nematophilum* resistant *C. elegans.* Since these results indicated which gene

transcript abundances were decreased in resistant worms, we hypothesized that one, or possibly many genes from this list would allow the *M. nematophilum* to infect a wild type *C. elegans*.

Three of the 33 genes identified by the microarray are lipl genes, encoding liapase products. These three genes were tested for function in mediating sensitivity to *M. nematophilum* in the growth assay. The gene *bus-1*, which has been previously shown to be important in mediating the response to *M. nematophilum* and that was likewise identified in the microarray dataset, was included as a positive control. The RNAi knockdown and mutant assays indicates that two of the lipl genes (*lipl-4* and *lipl-3*) impacted worm growth in response to *M. nematophilum*. Refer to Table 1 for results of RNAi knockdown assays.

Table 1.

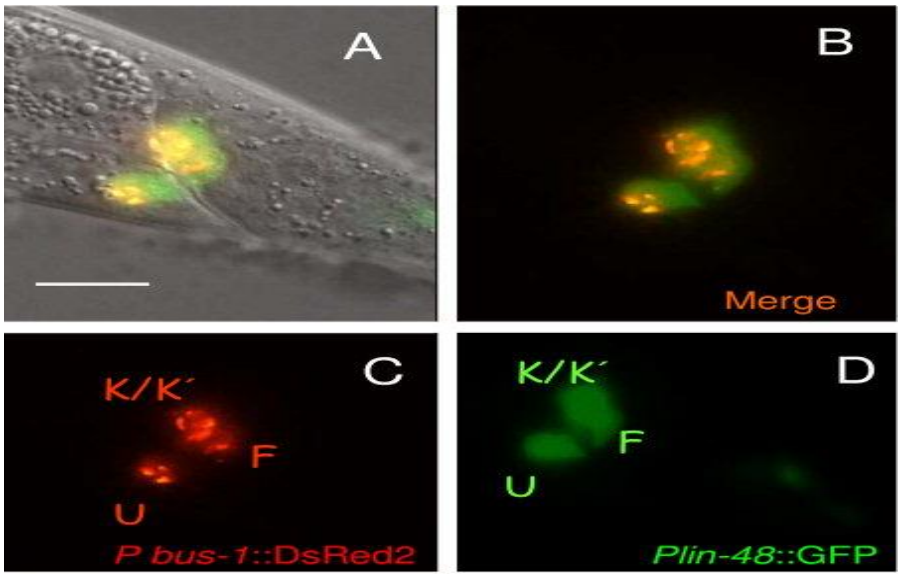
Experimental Gene	Control without *M. nematophilum*	Control with *M. nematophilum*	Experimental without *M. nematophilum*	Experimental with *M. nematophilum*
***lipl-5* mutant**	78.26%	2.00%	85.33%	7.84%
***lipl-5* (ZK6.7) RNAi**	75.62%	7.07%	95.35%	6.25%
***lipl-5* (ZK6.7) RNAi** *different strain	12.92%	6.89%	20.65%	2.00%
***lipl-4* mutant**	117.39%	14.81%	125.00%	**123.08%**
***lipl-4* (K04A8.5) RNAi**	142.37%	12.28%	59.66%	0
***lipl-3* (R11G11.14) RNAi**	62.04%	8.16%	121.24%	**16.85%**
***bus-1* mutant**	108.93%	20.98%	131.43%	**81.98%**

**Results in a yellow box indicate statistically significant results where worms with RNAi knockdown grew to an L4 stage faster than wild type worms on a lawn of *M. nematophilum* with a p-value ≤ 0.05 from a double sided z-test.

From the RNAi knockdown results, which indicated *lipl-4*, *lipl-3* and *bus-1* mutants were able to grow statistically better in the presence of *M. nematophilum* than wild type *C. elegans*, we

created GFP transgene worms. These specific genes were tagged within a wild type worm in order to determine specifically where these genes are translated within the nematode. Upon putting these worms under a florescent microscope, only the *bus-1* gene product was specifically expressed within the hindgut. The other tagged proteins were translated in many areas within the *C. elegans.* These results indicate that the *lipl* genes may impact *M. nematophilum* sensitivity, but are not necessarily the direct targets for EGL-38 in hindgut cells. Below, in Figure 2, is a picture of a wild type worm with *bus-1* proteins tagged under florescence taken from the literature (Gravato-Nobre & Hodgkin, 2008). My evaluation of *bus-1::gfp* expression yielded a similar result.

Figure 2.

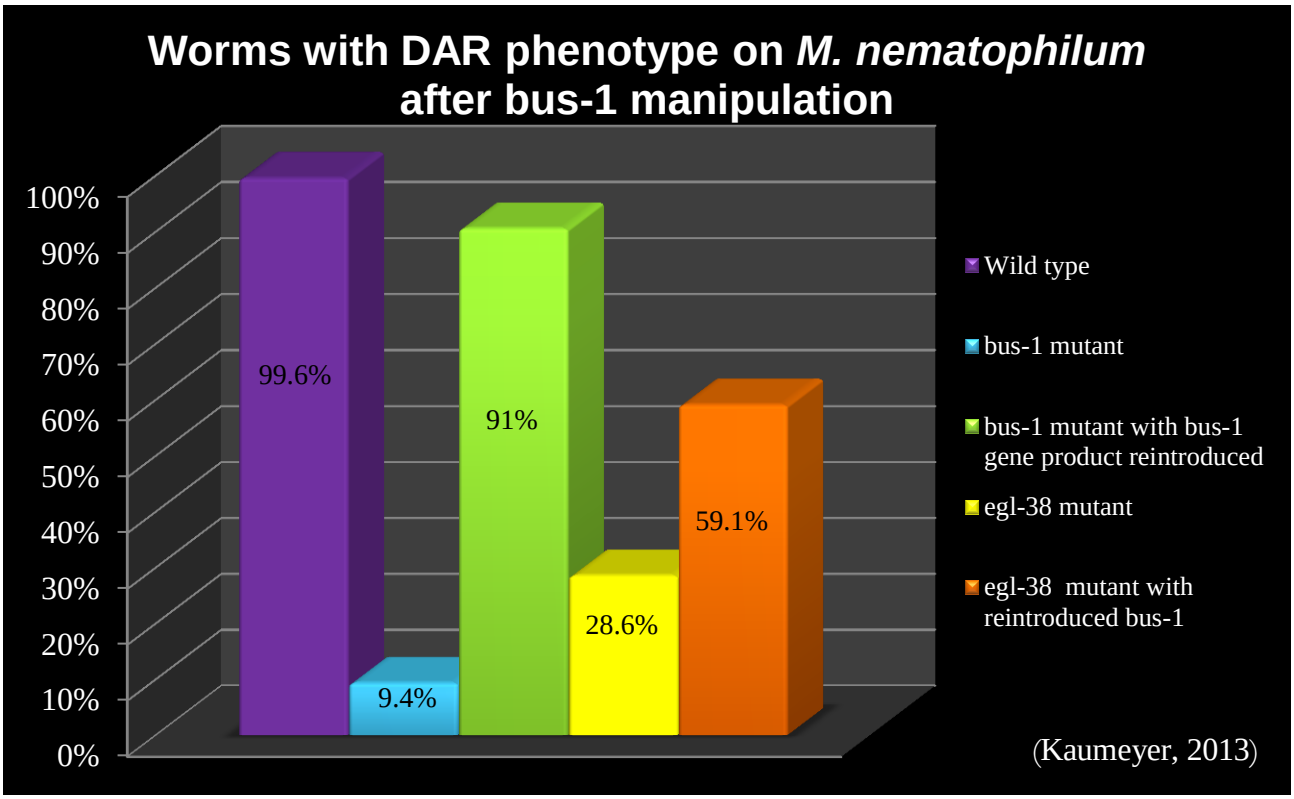


Picture **A** shows where GFP of both *bus-1* and *lin-48* (also a target of *egl-38*) are expressed in the hindgut of the *C. elegans.* Picture **B** shows both *bus-1* and *lin-38* GFP merged together, while pictures **C** and **D** show specifically *bus-1* GFP and *lin-48* GFP, respectively.
(Gravato-Nobre & Hodgkin, 2008)

Finally, in order to determine the importance of *bus-1*, specifically in the host/pathogen interaction, a lab colleague (Benjamin Kaumeyer) introduced *bus-1* into an EGL-38 and *bus-1* mutant worm under the control of a different promotor, *egl-5*. The presence of the *egl-5* promotor eliminated the ability of *egl-38* to impact the *C. elegans* response to the *M. nematophilum.* The introduction of the *bus-1* gene product proved to be enough to significantly restore the sensitivity

to the *bus-1* mutant worms (Kaumeyer, 2013). The introduction of *bus-1* into egl-38 mutant worms did restore sensitivity to the majority of the worms, however did not restore sensitivity to the extent of the *bus-1* mutants (see Table 3 below). This result supports that the protein product of *bus-1* is sufficient enough and able to rescue the transcription factor, *egl-38*, but that there are other genetic players involved in regulating this host/pathogen interaction. However, these results argue that *bus-1* is a key hindgut target for EGL-38 in mediating response to *M. nematophilum* within *C. elegans*.

Table 3.



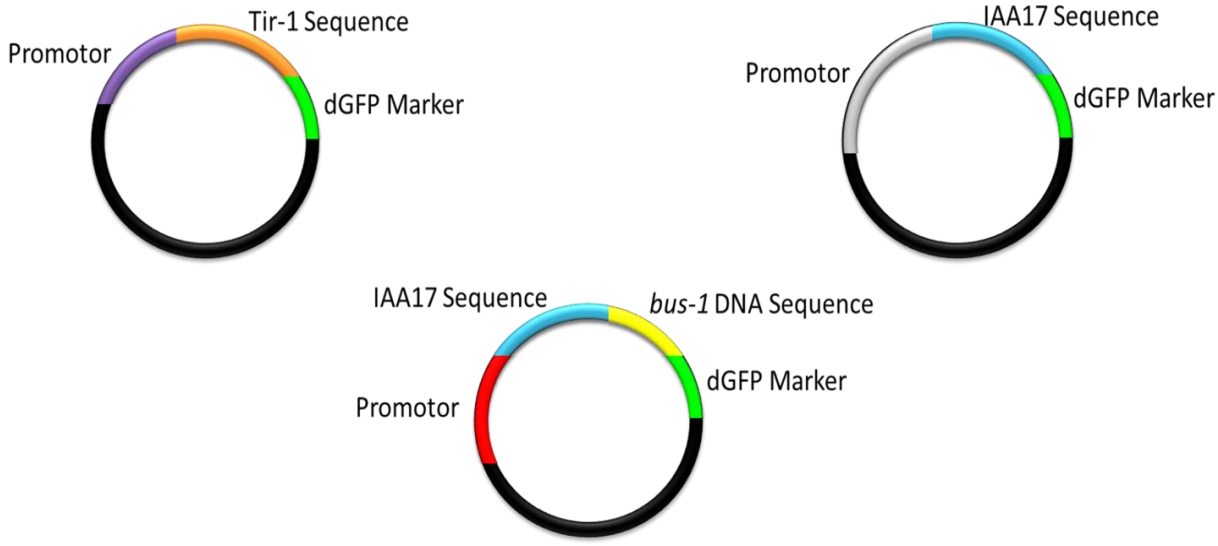
Due to the results obtained in this aim, we hypothesize that *bus-1* is an important EGL-38 dependent contributor in the *C. elegans* genome that mediates the host/pathogen interaction of *C. elegans* and *M. nematophilum.* However, *egl-38* mutants have many developmental defects. This information causes new research questions to arise regarding whether the loss of bus-1 is primarily developmental, or whether it plays a physiological role in mediating the *M. nematophilum* response. To address this question, we have asked the following questions: Is it possible to 'cure' an infection of *M. nematophilum* by silencing the gene products of bus-1? When developmentally

is bus-1 needed to allow a DAR phenotype to form? Could we design a way to essentially turn the bus-1 gene products on and off within a *C. elegans?* In order to find answers to these new questions and to physiologically discern the implications of bus-1, we are generating and implementing an inducible protein degron via a plant hormone, Auxin.

RESEARCH AIM 2

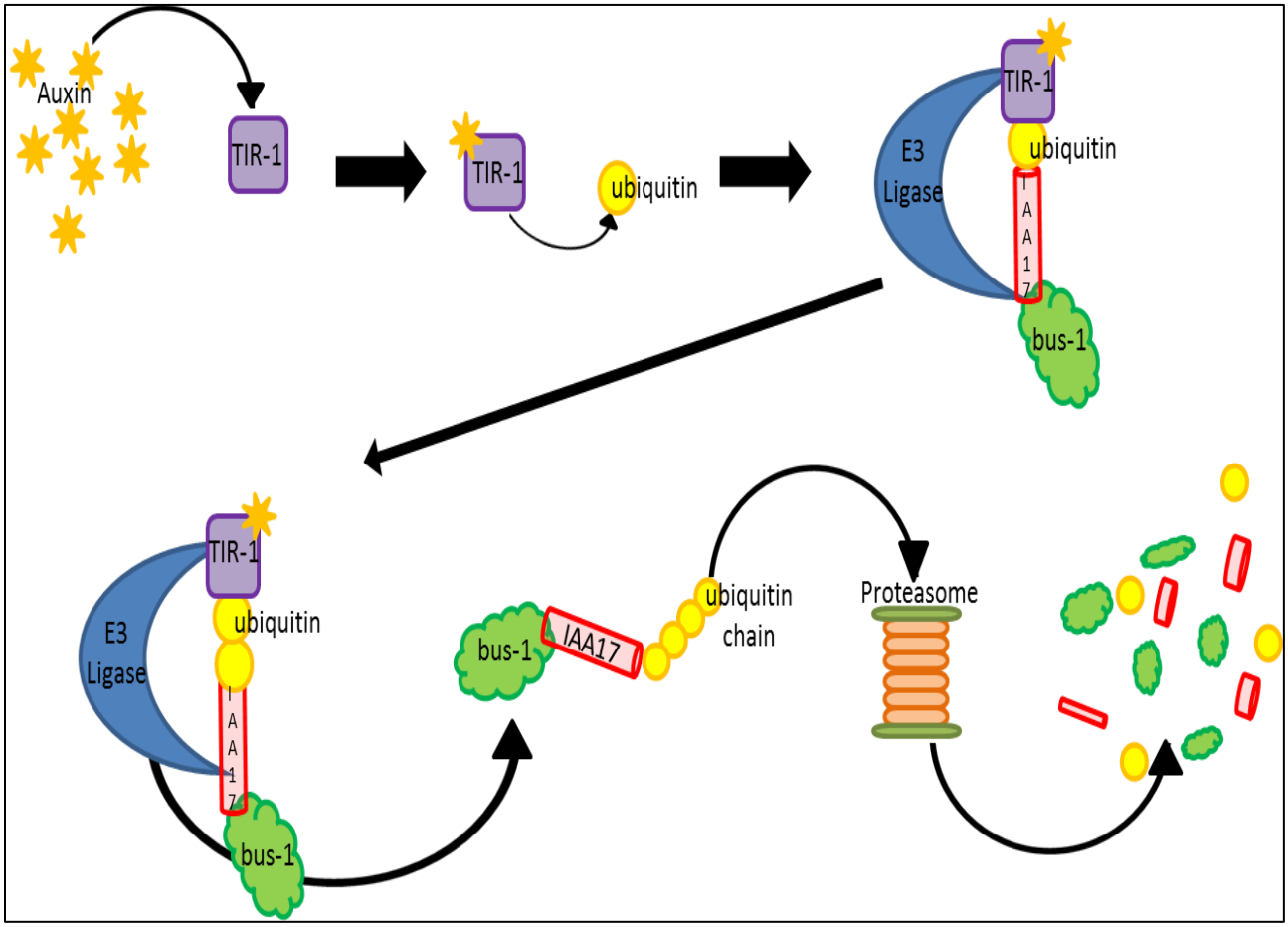
Auxin, indole-3-acetic acid, is a hormone found in plants and is necessary for embryo development and for fruit and leaf abscission. Auxin naturally acts as an 'inhibitor of inhibitors' within plant hormones. In a normal plant system, auxin enters a plant cell and binds to a protein receptor called TIR-1 and activates the protein to attract ubiquitin. A stem cell factor (SCF) E3 ligase enzyme is then used by the cell to transfer ubiquitin from the TIR-1 protein to an IAA17 protein. Under normal circumstances, within a plant cell the IAA17 proteins bind to response factors in order to inhibit promotors within the plant cell. Therefore, within a plant cell, increased auxin levels inhibit the cell's natural inhibitors, increasing transcription. If auxin levels decrease, transcription therefore decreases. (Kimball, 2013)

Non-plant organisms do not produce auxin, TIR-1 or IAA17 proteins. However, non-plant organisms do share a similar SCF pathway (De Lartigue, 2011). Nishimura et al. were able to design a protocol introducing TIR-1 and IAA17 into yeast, human, monkey and chicken cell lines (2009). We are using their work as a base to design and implement this type of protocol in order to move this system into a living model system, *C. elegans*. In order to move this protocol from cell lines and yeast into *C. elegans* we designed 3 specific plasmids (as seen below). One plasmid has the sequence to encode for the TIR-1 protein, another plasmid contains the sequence that encodes for the IAA17 proteins and the final plasmid that contains the IAA17 sequence attached to the bus-1 gene sequence.



This protocol employs the SCF degradation pathway in order to break down proteins tagged with an Auxin, IAA17 sequence (Nishimura et al., 2009). *bus-1* mutant nematodes would be injected with plasmids to encode for the TIR-1 binding complex as well as a plasmid to encode for an IAA17 tagged *bus-1* sequence. This step would ensure the only *bus-1* protein products within the nematode would have an IAA17 tag, in order to completely degrade all *bus-1* proteins within the nematode. A mechanism for the degrodation of *bus-1* protein products via Auxin and the SCF pathway can be seen below in Figure 3.

Figure 3.



**Upon the addition of Auxin to the environment of the *C. elegans* and *M. nematophilum*, the Auxin will activate TIR-1 inhibitor proteins. These activated TIR-1 proteins will bind to ubiquitin. The TIR-1, with the help of an E3 ligase will transfer the ubiquitin to any protein with an IAA17 DNA sequence. After a chain of ubiquitin has been added to the *bus-1* protein via an IAA17 sequence, a proteasome will breakdown the tagged protein, eliminating that protein.

Aim 2 Methods

This section of the research is still a work in progress. The first step of this section was to ensure the presence of auxin and ethanol (the solvent for the auxin) did not negatively affect the development and growth rate of the *C. elegans.* In order determine this; controls were set up, involving a total of 12 environments. See Table 4 below to view all controls and conditions established and tested.

Table 4.

OP50 bacterium with wild type worms	OP50 bacterium with bus-1 mutant worms	*M. nematophilum* with wild type worms	*M. nematophilum* with bus-1 mutant worms
Ethanol only	Ethanol only	Ethanol only	Ethanol only
M9 control	M9 control	M9 control	M9 control
Ethanol and Auxin	Ethanol and Auxin	Ethanol and Auxin	Ethanol and Auxin

All of these conditions were important to establish in order to guarantee that any change in growth or phenotype observed would be due to plasmids injected into the worm's genome, not due to the environment of the worms. Each assay was conducted as the earlier assays, including a two day time period for the *C. elegans* eggs to grow and then counting the amount of organisms to reach an L4 adult stage on day 3.

The plasmids needed for these experiments were generated from samples received from Dr. Yoshinobu Kaneko's lab at Osaka University. These plasmids contained the sequences for the IAA17 sequence as well as the TIR-1 sequence that were used in the published paper by Nishimura et al. (2009). Nishimura et al. determined which specific sequences and lengths are sufficient enough to induce the degradation response. The paper identified two lengths that were sufficient for the IAA17 response. In order to ensure the plasmids generated for the *C. elegans* had all the necessary amino acids, for each plasmid that needs the IAA17 sequence, there are two different

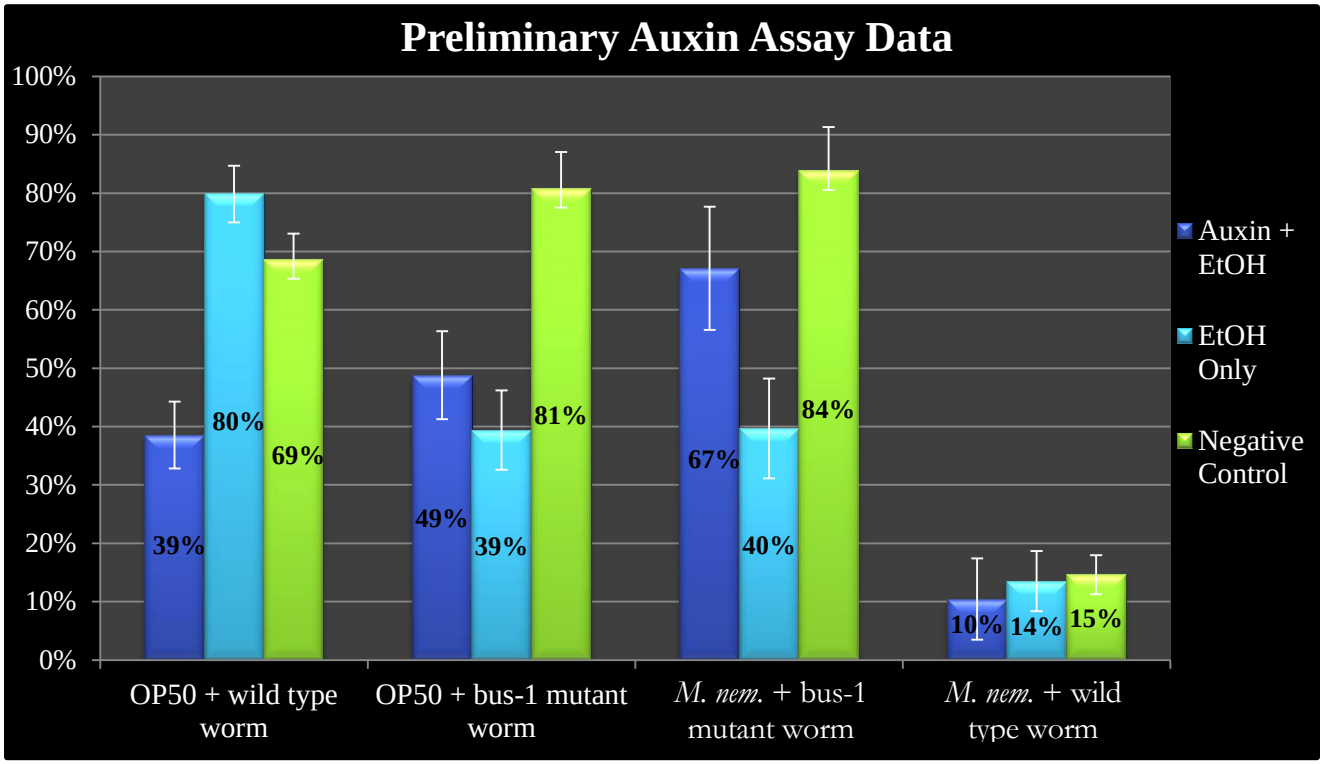
plasmids with a 'short' and 'long' IAA17 sequence, respectively. These five plasmids were generated via plasmids digests, PCR amplification, ligation and transformation into DH5α cells. In order to determine that there were not any mutations within the sequences, each plasmid was sequenced and made into an injection mix to be injected into the *bus-1* mutant *C. elegans* to create lines to be used in the Auxin protocol assays. As of this point in time, all plasmids have been generated and sequenced accept for the one plasmid including the TIR-1 sequence.

The TIR-1 plasmid as well as the IAA17 plasmid that does not contain the *bus-1* gene sequence would be injected into the *C. elegans* and used as a control to ensure that the presence of both the TIR-1 and IAA17 gene product within the nematode would not affect the worm's growth, health or phenotype. The experimental conditions will include injecting the TIR-1 plasmid and the plasmid containing IAA17 as well as the sequence for the *bus-1* gene product into a *bus-1* mutant *C. elegans.* By injecting these plasmids into a mutant worm, one can be assured that the only BUS-1 proteins present within the worm are tagged with the IAA17. This would assure that upon the addition of the Auxin hormone, there would be 100% BUS-1 protein degradation within the *C. elegans.*

Aim 2 Results

Due to the ongoing nature of this section of my research, only preliminary data can be accounted for. Below (Table 5) is the data collected when testing the environment controls. It is important to note that these assays have only been replicated twice, so these findings are simply early results. However, although this data is of a small sample size, the trends observed are similar to what would be expected. As can be seen in Table 4, the environments with the *bus-1* mutants and wild type worms without the presence of *M. nematophilum* grew noticeably more so than the environment containing wild type worms and *M. nematophilum.*

Table 5.



Although this research is still being conducted, the preliminary assay results indicated that the worms are able to survive and do not develop unexpected phenotypes on plates with a higher concentration of EtOH and auxin than we presume will be necessary for these experiments. These results also indicate that the presence of EtOH and auxin do not interfere with the bacterium's ability to infect the *C. elegans* and cause the DAR phenotype. Once all the plasmids are generated and worm lines created, more assays involving what stages *bus-1* is necessary for normal development and whether or not derogating bus-1 can 'cure' a *C. elegans* and the DAR phenotype can be conducted.

Aim One and Two Discussion

Results determined through the microarray analysis conducted by Jia, Johnson and Chamberlin (unpublished) implicated a list of genes that may be the cause as to why, on a genomic level, *egl-38* is necessary for the sensitivity of *C. elegans* to *M. nematophilum.* Through a series of RNAi knockdown, genetic mutant and gene transcriptional reporter assays *bus-1* was implicated as a main genetic contributor to the interaction between the host and pathogen. To further support the results that *bus-1* was the main player in this interaction, upon the reintroduction of *bus-1* gene product in an *egl-38* mutant worm, sensitivity and a DAR phenotype were observed in the presence of *M. nematophilum (*Kaumeyer, 2013*).* The results of Aim #1 did not identify additional critical genes; therefore, we concluded that *bus-1* is the primary EGL-38 target gene responsible for the pathogen's ability to infect the nematode.

Once all five Auxin plasmids are prepared, they will be injected into *bus-1* mutant *C. elegans* in order to create lines of worms where the plasmids are incorporated into the germ line of the organisms. Therefore, the only *bus-1* gene products present in the worm will have an IAA17 tag that will be targeted for degradation in the presence of Auxin. The implications and uses of this technology are endless. Time assays could be conducted to determine how long *bus-1* is necessary to allow an interaction to occur. Assays could be done to determine when, developmentally, BUS-1 needs to be present or absent to form specific phenotypes. Tests could be conducted to determine if it is possible to turn on and off *bus-1* via the presence of Auxin to determine the effects it would have on the worm's physiology and phenotype. Differing concentrations of Auxin could also be used in order to lessen but not completely remove BUS-1 to determine how active *bus-1* must be in order to cause a DAR phenotype. Most importantly, once this protocol is established, this could be used for a multitude of different genes for differing focuses of study. Since this would establish a protocol where auxin was incorporated in an organism other than cell lines and yeast, in the future, it may be possible to alter this protocol in order to be used in other, more complicated model systems.

Acknowledgements

I would like to extend my deepest thanks to Dr. Helen Chamberlin, my research advisor for all of her help, knowledge and support over the two years I have spent in her lab. I would also like to thank the graduate and undergraduate students, as well as the lab assistant in the Chamberlin lab for their insights, support and friendship. I would also like to extend a thank you to my research committee, Dr. Chamberlin and Dr. Dawes for their time, guidance and for the opportunity to present my work. Finally, thank you to all the institutions and scholarships that have funded my education over the past four years; including Choose Ohio First (FSO), Pelotonia, The Ohio State University Arts and Sciences, The Ohio State University Biological Sciences and Dr. Mayers Summer Research scholarships.

References

Darby, C. (2005) Interactions with microbial pathogens, WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/.

De Lartigue, Jane. (2011) "The SCF/KIT Pathway's Roles: Interest in Therapeutic Targets Is Growing." OncLive (2011). OncLive. Intellisphere, LLC. Web. 1 May 2015.

Gravato-Nobre, Maria J., and Jonathan Hodgkin. 2008, Dec. "The Acyltransferase Gene Bus-1 Exhibits Conserved and Specific Expression in Nematode Rectal Cells and Reveals Pathogen-induced Cell Swelling." (2008) Developmental Dynamics 237.12 (2008): 3762-776. Wiley Online Library. Wiley. Web. 1 May 2015.

Jia, Johnson and Chamberlin (2009) unpublished manuscript. Located at: Chamberlin private collection at The Ohio State University.

Kaumeyer, Benjamin (2013). "The Role of Egl-38/Pax in a C. Elegans Hindgut Infection." The Ohio State University Knowledge Bank (2013). Web. 1 Sept. 2014.

Kimball, John W. (2013) "Auxin." Auxin. The Saylor Foundation, 2 Jan. 2013. Web. 17 May 2015. <http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/Auxin.html#abscission>

Nishimura, Kohei, Tatsuo Fukagawa, Haruhiko Takisawa, Tatsuo Kakimoto, and Masato Kanemaki. (2009) "An Auxin-based Degron System for the Rapid Depletion of Proteins in Nonplant Cells." Nat Meth Nature Methods 6 (2009): 917-22. Nature Methods. Web. 4 Oct. 2014.

"WormClassroom." (2013) A Short History of C. Elegans Research. Laboratory for Optical and Computational Instrumentation at the University of Wisconsin-Madison. Web. 1 May 2015. <http://wormclassroom.org/short-history-c-elegans-research>.

Photo Credit:

Caenorhabditis elegans hermaphrodite adult (2013) [Internet].[cited 2014 Feb 27] . Available from: http://en.wikipedia.org/wiki/File:Caenorhabditis_elegans_hermaphrodite_adult-en.svg